CHARACTERISATION OF BACKGROUND BIOLOGICAL AEROSOL

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Sampling of ambient air using a glass cyclone system for fluorescence background determination is described. Twice weekly samples were taken at the University College Galway's atmospheric research field station at Mace Head, on the west coast of Ireland. The work is undertaken in collaboration with the Aerobiological Laboratory at ERDEC,MD. The bioaerosol sampling system and procedures are described in this first Interim Report. Use is made of a fluorescence protocol developed in the ERDEC Laboratories.

Preliminary analyses have also been carried out of the natural background aerosol particle size distribution, measures using a TSI aerodynamic particle sizer,at the same time as the bioaerosol sampling. Representative measurements of background aerosol size, surface and volume distributions for clean marine air and for polluted air at the Mace Head site are also presented.

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CHARACTERISATION OF BACKROUND BIOLOGICAL AEROSOL

1. Introduction

Natural fluorophores occurr intriniscally in all biological molecules. For proteins, there are three intrinsic fluors- tryptophan, tyrosine and phenylalanine. Tryptophan is the most highly fluorescent amino acid and in general accounts for 90% of the total fluorescence in proteins. This natural fluorophore is highly sensitive to its surrounding environment (ie., solvent, pH, and presence of a quencher, a small molecule, or a neighbouring group in the protein). Proteins absorb light near 280 nm, and fluorescence emission maxima range from 320 to 350 nm. Fluorescence lifetimes of tryptophan residues range from 1 to 6 nsec. In general the nucleotides and nucleic acids are nonfluorescent with the exception of yeast $tRNA^{PHE}$ which contains a highly fluorescent base known as the Y-base. This has an emission maximum near 470 nm and a lifetime near 6 nsec. The reduced nicotinamide adenine dinucleotides (NADH, NADHP) are highly fluorescent with absorption and emission maxima at 340 and 450 nm, respectively. Riboflavin, FMN (flavin mononucleotide), and FAD (flavin adenine dinucleotide) absorb light in the visible range ($\cong$ 450 nm) and emit around 515 nm. Typical lifetimes for FMN and FAD are 4.7 and 2.3 nsec respectively.Bacterial spores contain significant amounts of flavins and nicotinamide compounds. Viruses and protein toxins commonly contain one or more of the fluorescent amino acids.

The collection of biological aerosol for fluorescence background determination at the background site at Mace Head, Galway, on the west coast of Ireland has been ongoing since March 7th, 1996. This work has been undertaken in close collaboration and contact with the Aerbiological Laboratory at ERDEC, under group leader Patrick Nolan with Hugh Carlon, Dorothea Paterno and Sarah Cork. In addition Kate Ka Ong, Steve Christesen, Dougles Somerville and Donna Carlile have provided support for fluorescence analysis.

2. Bioaerosol Sampling Using a Glass Cyclone Sampler

Effective biological analysis of airborne particles requires samplers operating at a high flow rate and with the capability of concentrating the air particulate into a fairly small liquid volume. The glass cyclone sampler fulfills these two requirements. The cyclone has collection fluid continuously sprayed into the sampler inlet and operates at flow rates of 500 L per minute.

The principle of operation, is based on the production of a fine liquid mist in a rapidly moving airstream with collection of the aerosol in the liquid which is collected at the cyclone outlet.

Since the original version of the cyclone was developed by Aerojet General Corporation, under Government contract, it became known as the Aerojet General cyclone. This cyclone sampler was further described by Buchanan et al. (1972). The airborne particles are removed from the airstream mainly through impingement into the liquid film formed along the cyclone walls, and then washed off into a collecting reservoir. The collection efficiency of the Aerojet General cyclone was measured by May et al. (1976) and more recent collection efficiency work by Upton et al. (1993) and Griffiths et al. (1993) extend the range of aerosol size up to 20 μm. A summary of collection efficiency values obtained for the glass cyclone sampler is shown in Table 1.

The glass cyclone sampler is available from the Hampshire Glassware Company (Griffiths & DeCosemo, 1994). Two such cyclones have been procured for this work. In addition a 1300 watt (1.75 hp) Electrolux variable speed vacuum cleaner, with the capability of aspirating air @ 500 L per minute is also used. A rotameter is placed in the suction line between the cyclone and the vacuum cleaner - this is considered to be a reliable and direct method of monitoring the flow rate. The sample port has been fitted with a 1 inch copper sheath with a 19 gauge hypodermic needle inserted through a small hole drilled in the centre of the sheath. The needle is bent in a slight curve for delivary of the sampling media (PBS-Phosphate Buffered Saline) into the centre of the entry port of the cyclone.

Table 1 **Collection efficiency values for the glass cyclone**

Bioaerosol Species	Mean diameter (1 μm)	Wind Speed ms^{-1}	Collection Efficiency(%)	Work references
S.marcescens	1	0(laboratory)	70	Decker et al (1969)
Bacillus subtilis var niger	0.9	0 (laboratory)	71	Buchanan et al (1972)
	1.8	0 (laboratory)	90	Buchanan et al (1972)
	3.6	0 (laboratory)	96	Buchanan et al (1972)
	20	1 - 6	66	May et al (1976)
	30	1 - 6	74	
S. cerevisiae	5 - 20	0 - 4	80 - 100	Upton et al (1993)

A digitally controlled peristaltic pump (manufactured by Ismatec, UK Ltd.) has the capability of delivering flow rates between 0.06 and 3.6 mL per minute when used with size 13 silicone tubing. The microprocessor controlled unit accurately permits a rate of 1 mL per minute. Two pump heads are used - one to deliver the buffer solution to the cyclone and - the other to deliver the collected sampler solution to the glass collecting bottle. A silicone tube attached to a secondary opening in the collection bottle is also attached to an opening in the cyclone for pressure equalisation.

It should be mentioned that with the prevailing relatively high humidity values (typically in excess of around 70%) at the background field station at Mace Head on the west coast of Ireland, that evaporative losses of buffer solution are fairly minimal. In addition, possible difficulties associated with sampling at relatively low temperatures are not anticipated at Mace Head since temperatures are rarely $< 32^{\circ}F$ because of the modifying influence of the Eastern Atlantic warm Gulf Stream.

3. Bioaerosol Fluorescence Measurement Method

The protocol for sterilisation and cleaning of sampler equipment is detailed in Appendix 1 of this interim report. Total fluorescence measurements of biological samples have been determined in University College Galway using a Perkin Elmer LS 50B.

Air samples collected for total fluorescence measurements must be analysed within 24 hours. As we are interested in autofluorescence and also to prevent the introduction of chemicals/interferents, sample pretreatment involves dilution into water or PBS (Phosphate Buffered Saline), only if necessary. The test concentration of the sample is determined using a uv/vis spectrometer. Scanning from 200-400 nm the sample concentration with an absorbance between 0.1 and 0.5 AU is chosen. Using the same dilution factor the LS 50B spectrofluorometer is programmed to perform mulptiple scans on sample and buffer using the following programme settings.

	Scheme I	Scheme II
Excitation	250-310 nm 4 nm increment	310-530 nm 20 nm increment
Emission	265-550 nm 1 nm increment	330-800 nm 1 nm increment
Width	2.5 nm	2.5 nm

Sterile, filtered, distilled water is also scanned under the same conditions as the water Raman signal provides a calibration standard for determining the fluorescence cross sections. The ratio of the water Raman peak area to the fluorescence peak area is proportional to the ratio of the water Raman cross section (in cm^2/molecule) to the fluorescence cross section (in cm^2/cfu). Therefore in each case the water spectrum is subtracted from the sample spectrum and the remaining peak is quantitised using the water Raman peak ratio. Specta are analysed using the Grams, Galactic Industries software programme.

Representative spectra obtained from bioaerosol samples with the glass cyclone system at Mace Head are shown in Figure 1 (Dr. Steven Christesen, private communication, 1996).

4. Background Aerosol Characterisation

Preliminary background field analyses of the natural aerosol particle size distribution measured (using a TSI aerodynamic particle sizer) over the same period as the biological sampling, have been carried out. Representative measurements of size distribution for marine air (background) measured on 23 May 1996 and polluted air masses measured on 30 May 1996 are shown in Figure 2. A comparison of the size spectra shows that the particulate number concentration dominates for the marine aerosol in the 1 - 10 μm diameter range as might be expected. Aerosol volume distributions for the two contrasting air masses are shown in Figure 3. Corresponding aerosol surface distributions in the form dS/dlogD ($\mu m^2/cm^3$) are shown in Figure 4 for the clean backround marine case and for the more polluted air case. Further analysis and interpretation of the aerosol measurements will be be presented in the following interim reports.

Figure 1

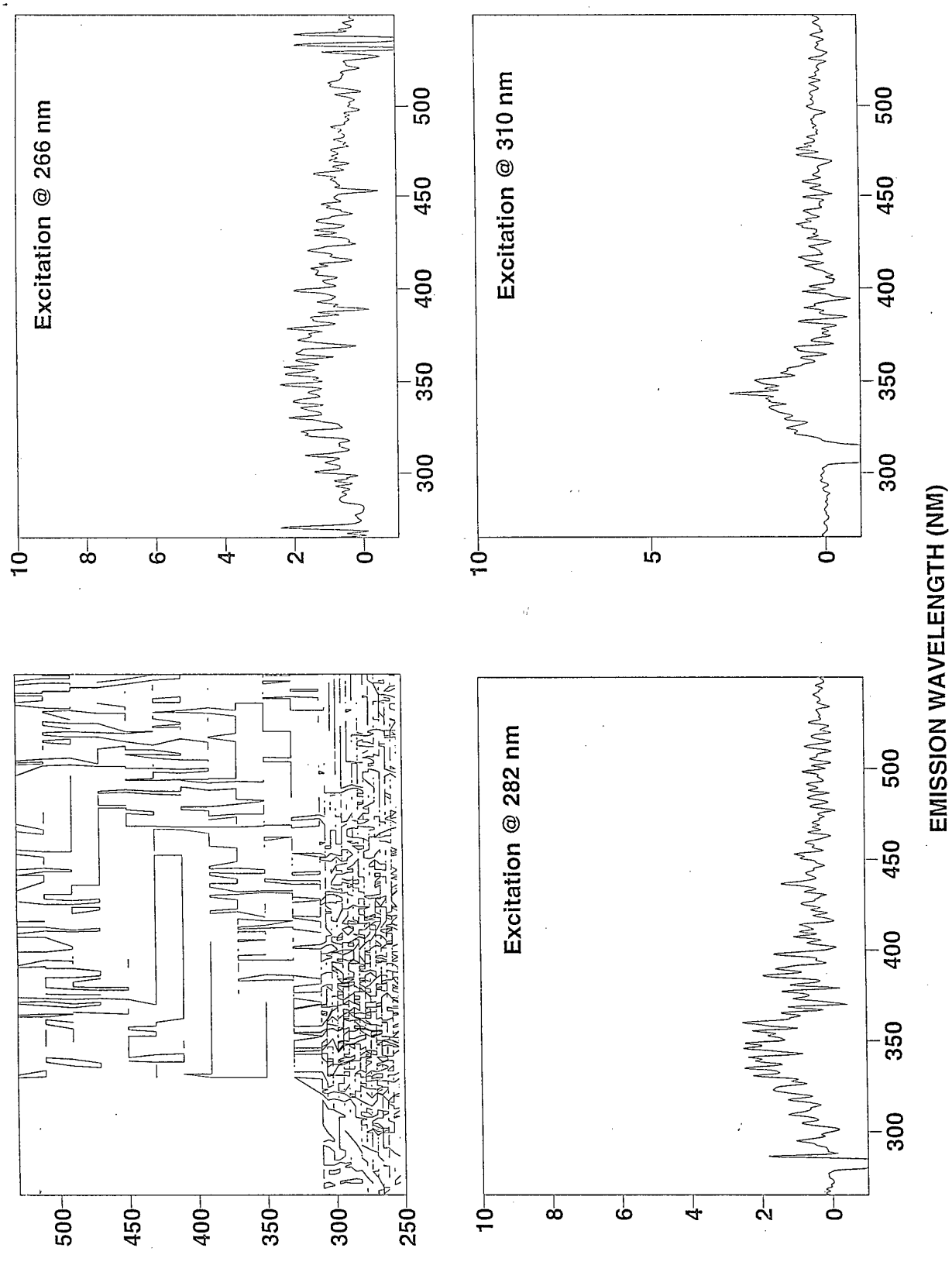
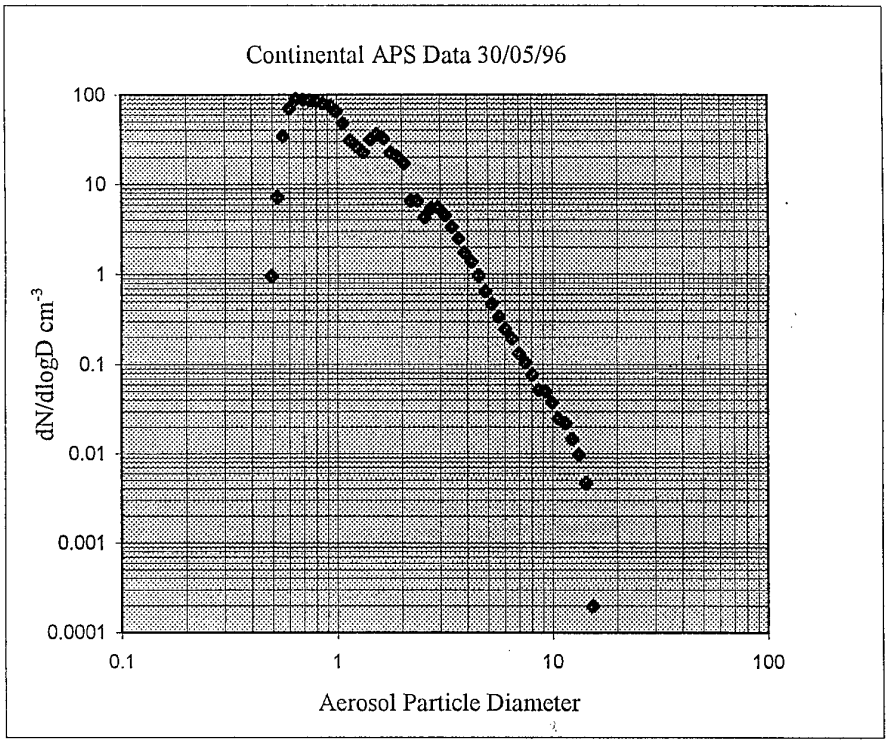

Excitation @ 266 nm
Excitation @ 310 nm
Excitation @ 282 nm
EX. WAVELENGTH (NM)
EMISSION WAVELENGTH (NM)
71096S with Water Subtracted

Figure 2


Continental APS Data 30/05/96
100
10
1
0.1
0.01
0.001
0.0001
dN/dlogD cm-3
0.1
1
10
100
Aerosol Particle Diameter

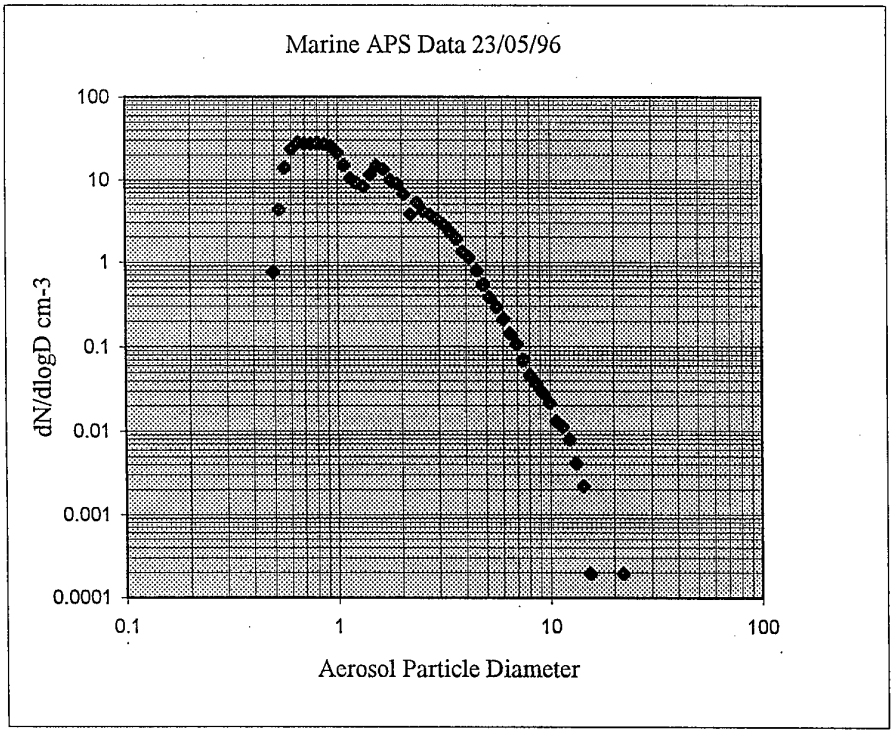
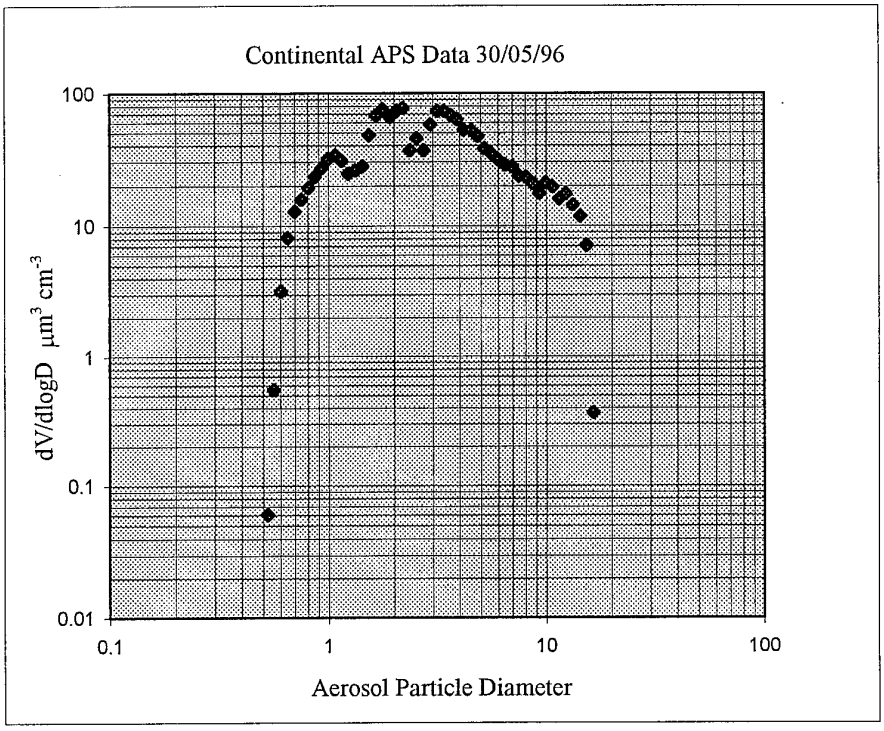

Marine APS Data 23/05/96
100
10
1
0.1
0.01
0.001
0.0001
dN/dlogD cm-3
0.1
1
10
100
Aerosol Particle Diameter

Figure 3


Continental APS Data 30/05/96
100
10
1
0.1
0.01
dV/dlogD µm³ cm⁻³
0.1
1
10
100
Aerosol Particle Diameter

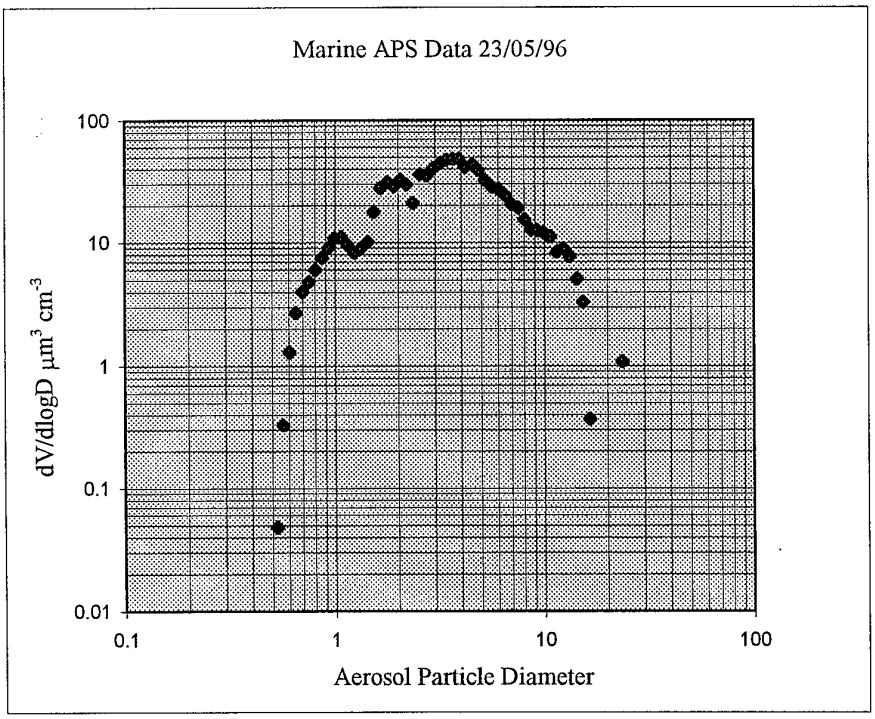
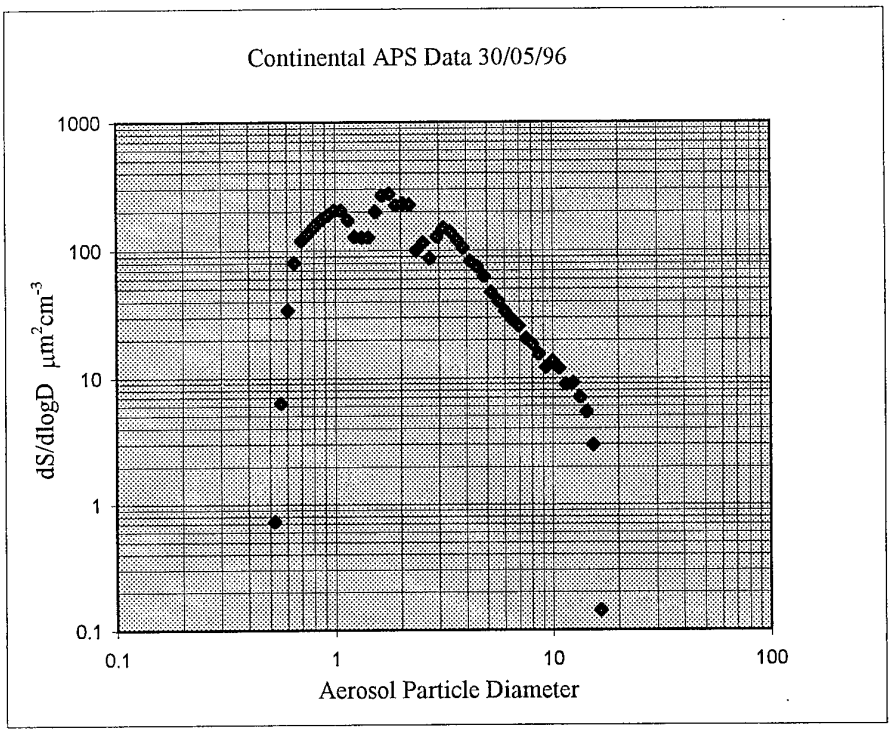

Marine APS Data 23/05/96
100
10
1
0.1
0.01
dV/dlogD µm³ cm⁻³
0.1
1
10
100
Aerosol Particle Diameter

Figure 4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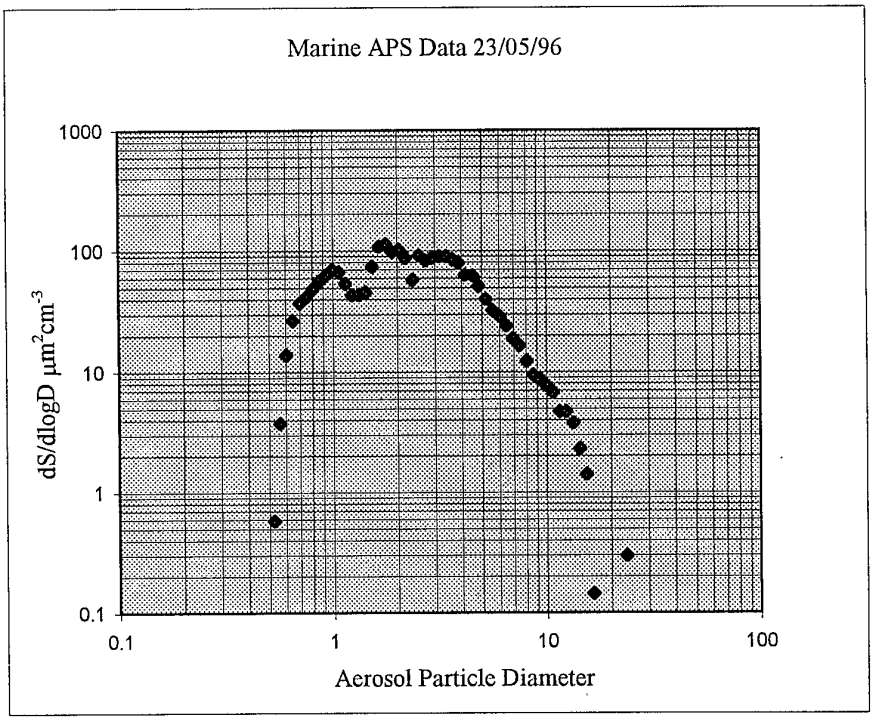
10
1
0.1
dS/dlogD μm²cm⁻³
0.1
1
10
100
Aerosol Particle Diameter


Marine APS Data 23/05/96
1000
100
10
1
0.1
dS/dlogD μm²cm⁻³
0.1
1
10
100
Aerosol Particle Diameter

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Griffiths, W.D., and DeCosemo, G.A.L., The assesssment of bioaerosols: a critical review. J. Aerosol Sci., 25, 1459-1492, 1994.

May, N.R., Pomeroy, N.P., and Hibbs, S. Sampling techniques for large windborne particles. J. Aerosol Sci., 7, 53-62, 1976.

Upton, S.L., Mark, D. Douglass, E.J., and Griffiths, W.D. The wind tunnel evaluation of the sampling efficiency of three biological aerosol samplers. Warren Spring Laboratory, unpublished report, U.K., 1993.

Appendix 1

The protocol for set-up, cleaning and sterilizing the glass cyclone sampler, used at ERDEC, as developed and written by Sarah Cork has been provided to University College Galway.

Materials

Autoclave

15% chlorine bleach in a capped 1 litre glass (Duran) bottle (x 2).

Sterile, filtered, distilled water (autoclaved at 121°c for 20 minutes and filtered through a 0.2 micron filter) in a capped 1 litre sterile glass (Duran) bottle (x 3).

Sterile strips of aluminium foil (autoclaved at 121°c for 20 mins).

Procedure

The cyclone, collection bottle, media bottle, tubing and hypodermic needle must be sterilised prior to any sampling day. After sterilisation non-sterile surfaces (including hands and lab benches) should not come in contact with the ends or insides of sterile items. All ends and ports of sterile items should be wraped in sterile aluminiun foil until assembly.

Plastic Media Bottle: Rinse with 1/2 litre of 15% chlorine bleach and allow a portion to exit through spout and tubing connector. Rinse bottle, spout and tubing connector with 250 ml (x 6) of sterile, filtered, distilled water, then wrap spout and tubing connector with sterile aluminium foil.

Collection Bottles and Glass Cyclone: Wash interior of bottles, bottle caps and cyclone with soap and water and rinse with tap water to remove soap residue. Rinse interior of items with 15% chlorine bleach, allowing bleach to run out of the cyclone's exit ports. Items are now sterile and should not come in contact with non-sterile surfaces. Rinse with sterile, filtered distilled water at least 6 times until items no longer smell of bleach, then cap bottles and cover all ports of the cycone with sterile aluminium foil.

Tubing and Hypodermic Needle: With tubing in place through peristaltic pump, place the entry end of the tubing in a container of 15% chlorine bleach. Place the needle and copper sheath into an empty container making sure that the tip of the needle is not touching non-sterile surfaces. Pump 50 ml of 15% chlorine through tubing and needle until tubing is completely filled with dilute bleach. Place entry end of tubing into sterile flask containing sterile, filtered, distilled water and pump 150 ml through tubing and needle. Follow the same procedure for tubing in second pump head. Wrap the copper sheath and tubing ends in sterile aluminium foil.

Sampling Procedure

Pour 300 ml of the sampling media into the plastic Nalgene media bottle. Sampling media for total fluorescence is phosphate buffered saline, pH 7.4 (Sigma Diagnostics, 120 mmol/L NaCL) which has been mixed with 1 litre of distilled water, filtered (through a 0.2 micron filter) and sterilized for 20 minutes ar 121°C. Do not tighten the cap on the media bottle in order to avoid suction building up inside. Attach entry end of tubing to the media bottle spigot and pump sampling media through the tubing until it completely displaces any water in the tubing and exits through the needle. Attach the copper sheath to the cycone. Attach collection bottle and equilibrium hose. Turn on vacuum set at 500 litres per minute and peristaltic pump set at 1 ml per minute. Begin sampling. At the end of the sampling period turn off the vacuum, undo equilibrium hose and tubing from collection bottle, cap and refrigerate sample as soon as possible.